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Review

Discovery and development of antiviral drugs for biodefense: Experience of a small biotechnology company

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Abstract

The unmet need for effective antivirals against potential agents of bioterrorism and emerging infections is obvious; however, the challenges to develop such drugs are daunting. Even with the passage of Project BioShield and more recently the BARDA legislation, there is still not a clear market for these types of drugs and limited federal funding available to support expensive drug development studies. SIGA Technologies, Inc. is a small biotech company committed to developing novel products for the prevention and treatment of severe infectious diseases, with an emphasis on products for diseases that could result from bioterrorism. Through trials and error SIGA has developed an approach to this problem in order to establish the infrastructure necessary to successfully advance new antiviral drugs from the discovery stage on through to licensure. The approach that we have taken to drug development is biology driven and dependent on a dispersive development model utilizing essential collaborations with academic, federal, and private sector partners. This consortium approach requires success in acquiring grants and contracts as well as iterative communication with the government and regulatory agencies. However, it can work as evidenced by the rapid progress of our lead antiviral against smallpox, ST-246, and should serve as the template for development of new antivirals against important biological pathogens.

Keywords: Antiviral; Drug development; Smallpox; Hemorrhagic fever virus; Category A; Biothreat agents

Contents

1. Introduction

Highly pathogenic viruses such as Ebola and variola pose a significant threat to human health, yet in most cases, therapies to prevent or treat these diseases are lacking. Project BioShield was put forward in 2004 by the U.S. President George W. Bush to help address this issue by expediting research and development of medical countermeasures against biothreat agents. In theory, this legislation gives the Food and Drug Administration (FDA) the ability to make promising treatments available quickly in emergency situations, and ensures that resources are available to pay for "next-generation" medical countermeasures. Project BioShield is a comprehensive effort overseen jointly by the Department of Health and Human Services (DHHS) and the Department of Homeland Security (DHS) with involvement from other federal agencies, including the Department of Defense (DOD), as appropriate. Recognizing the limitations of BioShield, additional legislation was passed in 2006 to help drug companies to bridge the "Valley of Death", the crucial middle phase of drug development between basic research and the acquisition of final products, which includes many of the late stage development activities required to support a New Drug Application (NDA). The Biomedical Advanced Research and Development Authority (BARDA) was created to facilitate col-

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laboration between companies and the federal government and to promote innovation. These measures are helpful, but there is still a significant disconnect between recognizing what needs to be done and actually accomplishing it in a timely fashion. We are committed to trying to bridge this gap. In the sections below, we will discuss the major challenges to develop these new antivirals and the approach we have taken for the development of new therapeutics against Category A viral biothreat agents.

2. Challenges to development of antivirals for biothreat agents

The first challenge that drug developers face is the paucity of available information about many of these exotic pathogens. Because these are primarily tropical diseases, endemic in developing countries, relatively little research attention and funding has been focused on them until recently. The hemorrhagic fever viruses are commonly lumped together into a group of "similar" diseases caused by four very different types of viruses: arenaviruses, bunyaviruses, filoviruses, and flaviviruses. While it is true that the clinical symptoms produced by these viruses are similar, each of the viruses has a different genome and replication strategy, so it is highly unlikely that a single drug will be developed that can treat all of these diseases.

Most of these pathogens require biosafety level 4 (BSL-4) containment, which is in short supply and has limited access. One alternative that is being explored is the use of surrogate viruses (e.g. Tacaribe instead of Junin, for the New World arenaviruses) that requires lower levels of bio-containment. This can be useful, but both granting and regulatory agencies consider the authentic pathogen as the "gold standard" for demonstrating therapeutic efficacy. A second alternative is the development of pseudotype virus assays or replicon systems, in which the envelope proteins of a pathogen enwrap a non-replicating genome expressing a convenient reporter gene, a "sheep in wolf's clothing". Although suitable for use in BSL-2 laboratories and amenable to high throughput screening, the limitation of these systems is that they are not live viruses in the truest sense and may not allow certain virus functions to be recapitulated as drug targets.

Work with the authentic agents requires BSL-3 or BSL-4 facilities, which are available in only a few locations in the U.S.: the United States Army Medical Research Institute for Infectious Diseases (USAMRIID), University of Texas Medical Branch (UTMB) Galveston, Southwest Foundation for Biomedical Research (SFBR) and the Centers for Disease Control and Prevention (CDC). Even more restrictive is the limited space available in which to conduct BSL-4 animal studies. This is a particular problem with non-human primates, which will likely be required for product licensure. Current facilities can only handle a small number of animals which limit the experiments that can be done and the statistical significance of the results obtained. Recognizing this problem, the National Institute of Allergy and Infectious Diseases (NIAID) is providing funding to build two new National Biocontainment Laboratories, one at Boston University and one at UTMB Galveston, both of which should be ready near the end of 2008. NIH is also building a new BSL-4 facility in Frederick, MD, next to USAMRIID at Fort Detrick that will be completed in 2008. The criteria for access to these facilities are not easily defined. The first and foremost requirement is money to fund the studies, followed by the scientists who are willing to work on the appropriate select agent and develop appropriate animal models. After that it is a matter of politics; what is the high profile agent of choice, is the particular government agency interested in it, have you proven that the small molecule is worthwhile and ready to be tested in animals? Insurance that these resources are effectively being used is of utmost importance.

As mentioned previously, there have been several animal models developed using surrogate BSL-2 and BSL-3 RNA viruses, but efficacy studies against the actual pathogens in BSL-4 will likely be required by the FDA for approval of a new therapeutic. Appropriate animal models will need to be developed and validated for each pathogen which will require finding the appropriate animal species and collecting enough natural history of infection to support their use in regulatory applications. Also, the chosen animal models will need to recapitulate human disease as closely as possible. This will involve obtaining disease information on infected humans, which is quite rare for some viruses; furthermore natural outbreaks of these diseases mainly occur in undeveloped countries which have limited surveillance and epidemiology capabilities. Another nuance of the animal models is the delineation of what point of intervention constitutes prevention versus treatment. Answers to these questions will greatly impact what indication a new antiviral drug receives from the FDA.

RNA viruses have relatively high mutation rates (around 1 per genome per replication event) because they lack proof-reading capacity in their replicases. In contrast, DNA viruses have considerably lower mutation rates (approximately 0.003 per genome per replication event) due to the proof-reading ability of DNA polymerases within the host cell. This trait predicts that RNA viral pathogens will be able to rapidly evolve resistance in the presence of antiviral drug selection. Thus, treatment for RNA pathogens may require combination of therapeutic modalities or the use of antiviral drugs that circumvent resistance, i.e., where induced mutations render the resistant virus less fit and unable to productively produce an infection. Combination therapy comes into play when the antiviral is used long term for chronic diseases such as the case of human immunodeficiency virus (HIV) treatment or in the event that the drug had to be given prophylactically for a long period of time. One would not expect acute use of an antiviral to produce significant resistance problems.

The clinical development pathway for antivirals against biothreat agents is convoluted, to say the least. Since most of these pathogens are not endemic in the United States and may be rare even in endemic areas, it is difficult to perform human efficacy studies with clinical rigor. Recognizing this problem, the FDA developed the Animal Rule (21 CFR 314.600). The FDA Animal Efficacy Rule (finalized May 2002) applies to the development/testing of drugs/biologicals to reduce or prevent serious/life-threatening conditions caused by exposure to lethal/permanently disabling toxic agent (chemical, biological, radiological, or nuclear substances), where human efficacy trials

are not feasible or ethical. Under this rule the FDA can rely on data from animal studies to provide substantial evidence of product effectiveness when: (1) there is a reasonably well-understood mechanism for the toxicity of the agent and its amelioration or prevention by the product; (2) the effect is demonstrated in either: more than one animal species expected to react with a response predictive for humans, or; one well-characterized animal species model (adequately evaluated for its responsiveness in humans) for predicting the response in humans; (3) the animal study endpoint is clearly related to the desired benefit in humans; and (4) data or information on the pharmacokinetics (PK) and pharmacodynamics (PD) of the product or other relevant data or information in animals or humans is sufficiently well understood to allow selection of an effective dose in humans, and it is therefore reasonable to expect the effectiveness of the product in animals to be a reliable indicator of its effectiveness in humans. Unfortunately, appropriate animal models for many of the Category A RNA viruses have not been defined or validated. Some of these diseases do occur at high frequency (e.g. Lassa Fever) so clinical studies in locations such as Africa may be necessary to support regulatory approval. Using animal efficacy studies to predict how an antiviral will work in humans is a challenge. The burden lies on the scientists' ability to recapitulate human disease in the animal, determine surrogate markers for viral action such as viral load, create the PK/PD link, and then convince the FDA that the drug will work based on this data.

The final, and in many ways most significant challenge, is funding late stage development of these antivirals products. This is often referred to as the "Valley of Death"; this is the crucial middle phase of drug development between basic research and acquisition of final products for which there is little available funding. This is also known as the critical path section of drug development by the FDA (Fig. 1). The NIH has recently implemented new types of contracts to try and bridge the gap between early stage research and filing a NDA. However, thus far these contracts do not cover typical Phase III human studies, if they are necessary. A related issue is the uncertainty of the market once the drug is successfully developed. Who will buy the drug and how much will be bought? Sizing the possible acquisition is very difficult—will it be based on military population, civilian population, or both? U.S. only, or a global market? As part of the regulatory process, companies have to prove that they can manufacture the drug product at 1/10 of the commercial size batch, but without knowing the commercial market this is at best an educated guess. This is the pharmaceutical equivalent of the "Field of Dreams"—if we develop it someone will purchase it. This is a difficult concept on which to base a viable business. Besides being biothreat agents, many of these diseases are endemic in developing countries where there is a real need for therapeutic drugs. Unfortunately, these countries cannot afford to pay for these drugs and the biotechnology industry cannot afford to provide them for free. Because of these market uncertainties, big pharmaceutical companies have not participated in this enterprise in any meaningful way. This is a problem that can benefit from the participation of agencies such as the World Health Organization (WHO) or the Gates Foundation.

3. SIGA's approach to drug development

SIGA is a publicly traded biotechnology company that has been engaged in the discovery, design, development, and commercialization of vaccines, antibiotics, and novel anti-infectives for the prevention and treatment of severe infectious diseases for the past 10 years. Since 2000, the focus of our research activities has been in the area of developing effective countermeasures against potential biothreat agents. For example, we have been successful in developing an antiviral against a disease that is no longer found in the environment, but is considered a major bioterror threat—smallpox (caused by variola virus). This antiviral, ST-246, has recently completed human Phase I multi-dose clinical trials and is on the track to obtain licensure in the next few years. This antiviral will also have utility against other pox viruses such as monkeypox as well as any emerging poxvirus diseases.

Because of the unique nature of these agents, and the size of our company, SIGA's drug development paradigm focuses on biological testing prior to performing extensive medicinal chemistry. Many large pharmaceutical companies can run high-throughput screens on roughly 50,000 compounds per day; they can then transfer hits to the chemistry department, where synthesis of hundreds of analogs is initiated. For many of these companies, the chemistry drives the drug development process. Other companies, like SIGA, first identify a hit, then fully characterize the compound's mechanism of action, potential cellular interactions, and pharmacokinetics before initiating chemistry efforts. At SIGA, compounds are screened in cell-based assays focusing on viral inhibition, cell toxicity, and mechanism of action. ST-246 was discovered using a cell-based screen on a diverse small molecule compound library using live vaccinia virus. Mechanism of action studies determined that ST-246 targets a protein responsible for egress and spread of the virus. ST-246 does not prevent replication, but does prevent disease (Yang et al., 2005). Because of this mechanism, it is possible to challenge and/or vaccinate in the presence of ST-246 and still elicit a protective immune response (Grosenbach et al., manuscript in preparation). This is important information that will enable the government to decide the appropriate countermeasures to use in the event of an outbreak.

Also taken into consideration is the biology of the disease model(s) and how a compound would appropriately interfere with this disease. In animal efficacy studies, we have shown that ST-246 can be given prophylactically, post-exposure prophylactically, and therapeutically to prevent and/or treat orthopoxvirus diseases (Quenelle et al., 2007; Sbrana et al., 2007) (Huggins et al., 20th ICAR, Palm Springs, 2007). Therefore, with ST-246, one could initiate treatment with the drug and then follow this with a vaccination campaign until the threat is gone.

Once the biological relevance of a compound series is determined, only at this point is medicinal chemistry initiated using all the collected biological data. Since many of these pathogens require high level containment, it is essential to ensure a potential lead compound has been thoroughly evaluated before efficacy testing begins. This means acceptable formulations, solubility,

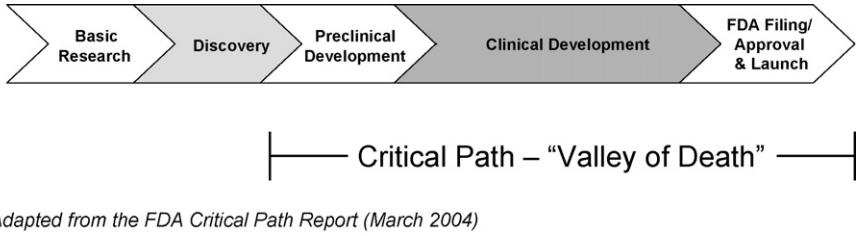

Fig. 1. The critical path for medical product development. Shown is an outline of the steps involved in the drug development process which can take 10–15 years from start to finish.

stability, and pharmacokinetic parameters as well as tolerability in appropriate animal species.

A dispersive development model is also necessary for moving these types of antivirals through development, especially animal efficacy testing (Fig. 2). We have set up essential collaborations with appropriate academic laboratories, federal officials, private facilities, and the Department of Defense to capture all the expertise required to study and test antivirals. In the case of smallpox, there are numerous surrogate animal models being studied across the United States, and elsewhere, and we have availed ourselves of as many of those animal models as possible to address potential FDA concerns. Also, access to certain viruses, such as variola virus, are highly restricted and work can only be done at limited sites under high level containment. In the case of variola virus, work can only be done in the BSL-4 laboratory at the CDC. Without all these collaborations, it would not have been possible to develop ST-246.

Funding all of these studies is very costly. To that end, we have sought and continue to seek grants and contracts to support development of our antiviral products. To date, we have received approximately $31 million to support discovery and development of ST-246 and this funding will support development though filing a NDA in 2009. This funding came from the National Institutes of Health (NIH), the Office of Biodefense Research Affairs (OBRA), and the Defense Threat Reduction Agency (DTRA). Similar levels of funding will be necessary to develop each individual antiviral product.

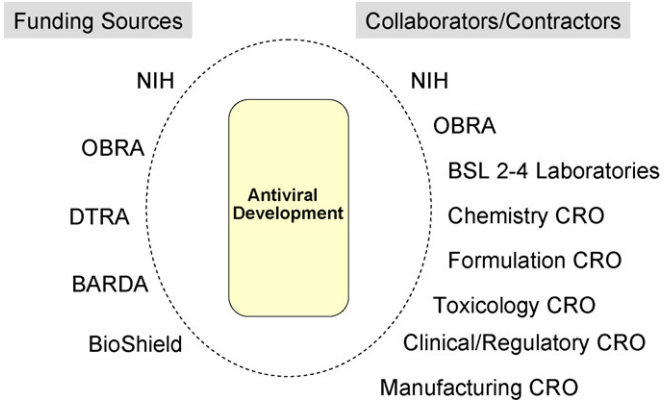

Fig. 2. Dispersive development model at SIGA. The diagram delineates SIGA's government funding sources and the collaborators and contractors necessary to develop countermeasures against potential agents of bioterrorism. CRO: Contract Research Organization.

Good and iterative communication with regulatory agencies, who will help define the studies that will be integral parts of the IND and NDA applications, is important. Biological countermeasure development is a relatively new area, so SIGA has repeated discussions with the agencies in order to determine the best path forward. These agencies can facilitate the design of the safety and toxicology studies, development of the appropriate animal models that will ultimately support the Animal Rule, and provide guidance on the clinical studies that will be required for licensure. These agencies also weigh in on desired dosing regimens, packaging, and potential markets for the drug. It is also beneficial to communicate with the Federal government and the military to let them know what drugs you have in the pipeline and their stage in development in the event there is an unexpected crisis.

As a small company, we cannot afford to have all of the necessary staff in place in-house. Rather we have built a network of company personnel, consultants, contractors, and government officials that can effectively work together to advance products through the pipeline. We plan to utilize this network to finish development of ST-246 and continue development of new antivirals against important viral pathogens.

4. Conclusion

Developing antivirals for potential bioterror agents and emerging pathogens is difficult for a small company, but it is also very important. Endemic viral diseases wreak havoc in developing countries, are emerging in new locations and continue to exist as biowarfare threats. We have had a number of recent examples of the rapidity with which an emerging viral disease can impact mankind. HIV was first reported in the United States in 1981, and now there are almost a million people in this country, and some 40 million in the world, who are living with HIV/AIDS. Other more recent emerging diseases are SARS (severe acute respiratory syndrome), and avian flu, which have the potential to cause major pandemics.

To successfully fight these existing and emerging pathogens SIGA is following a biology-driven approach which focuses initially on the interaction between the pathogen and small molecule inhibitor. With the mechanism and utility of a compound series clearly defined, SIGA will move on to medicinal chemistry to increase potency and selectivity. SIGA relies on a number of collaborators and contract organizations for medicinal chemistry, formulation development, BSL-4 testing, IND

and NDA enabling toxicology, FDA interactions, clinical study design, and drug manufacturing activities. This development infrastructure is very important and requires experienced project management and iterative communication and is part of SIGA's dispersive development model.

None of these activities would be feasible for a small company without external funding. SIGA has established a proven track record developing ST-246 and has been able to leverage this support for development of its other antivirals. Parallel to this, the government has also realized the need for support and is developing strategies to bridge the "valley of death" and clarify the new therapeutic agents it would like to acquire. Taken together, these measures should enable a small company to be successful in developing new drugs against biothreat agents and to use the same infrastructure to develop anti-infectives against more traditional pathogens.

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